

WHAT IS CLAIMED IS:

1. A method useful to detect a pathogenic microbe, the method comprising the step of subjecting DNA extracted from said microbe or a cDNA equivalent thereof, to a polymerase chain reaction comprising primers adapted to produce a detectable amplicon from a gene responsible for the pathogenicity of said microbe, and measuring in real time the accumulation of said amplicon during said reaction.
2. The method according to claim 1, wherein the polymerase chain reaction is performed in the presence of probe that selectively binds said amplicon and incorporates a label detectable upon reaction of the probe with a 5' nuclease.
3. The method according to claim 1, for the detection of at least two different pathogenic microbes in a given sample, the method comprising the step of subjecting a sample comprising DNA extracted from said microbes, or a cDNA equivalent thereof, to a polymerase chain reaction comprising primers adapted to produce at least one detectable amplicon from at least one gene of each pathogenic microbe in said sample, and then measuring in real time the accumulation of said amplicons during the reaction.
4. The method according to claim 1, for the detection of at least one pathogenic microbe selected from total coliforms, *E. coli*, *E. coli* O157:H7, toxigenic *M.aeruginosa*, *G.lamblia*, and *C. parvum*.
5. An amplicon having a nucleotide sequence selected from the coding sequence:
  - (a) the region spanning residues 2574-2895 of the *lacZ* gene of *E. coli*;
  - (b) the region spanning residues 2673-2759 of the *eaeA* gene of *E. coli* O157:H7;
  - (c) the region spanning residues 1438-1559 of the *mcyA* gene of *Microcystis aeruginosa*;
  - (d) the region spanning residues 222-296 of the  $\beta$ -giardin gene of *G. lamblia*;

- (e) the region spanning residues 411-485 of the  $\beta$ -giardin gene of *G. lamblia*; and
- (f) the region spanning residues 583-733 of the COWP gene of *C. parvum*.
6. An oligonucleotide probe that binds selectively to an amplicon defined in claim 5.
7. An oligonucleotide probe according to claim 6, bearing a fluorophore detectable upon reaction with a 5' nuclease.
8. An oligonucleotide probe having a nucleotide sequence selected from SEQ ID Nos. 3, 6, 9, 12, 15 and 18.
9. An oligonucleotide primer adapted to amplify an amplicon according to claim 5.
10. An oligonucleotide primer according to claim 9, having a nucleotide sequence selected from SEQ ID NOs. 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16 and 17.
11. A method for detecting total coliforms including *E. coli* in a given sample, comprising the step of subjecting DNA extracted therefrom to a polymerase chain reaction incorporating primers having SEQ ID NOs 4 and 5, and a probe having SEQ ID NO. 6.
12. A method for detecting *E. coli* O157:H7 in a given sample, comprising the step of subjecting DNA extracted therefrom to a polymerase chain reaction incorporating primers having SEQ ID NOs 1 and 2, and a probe having SEQ ID NO. 3.
13. A method for detecting *M. aeuroginosa* in given sample, comprising the step of subjecting DNA extracted therefrom to a polymerase chain reaction incorporating primers having SEQ ID NOs 7 and 8, and a probe having SEQ ID NO. 9.
14. A method for detecting *G. lamblia* in a given sample, comprising the step of subjecting DNA extracted therefrom to a polymerase chain reaction incorporating

either (A) primers having SEQ ID NOs 10 and 11, and a probe having SEQ ID NO. 12, or (B) primers having SEQ ID NOs 13 and 14, and a probe having SEQ ID NO. 15.

15. A method for detecting *C. parvum* in given sample, comprising the step of subjecting DNA extracted therefrom to a polymerase chain reaction incorporating primers having SEQ ID NOs 16 and 17, and a probe having SEQ ID NO. 18.

16. A method for discriminating between microbes *G. lamblia* and *G. muris*, comprising the step of subjecting DNA extracted from a selected one of said organisms to first and second polymerase chain reactions adapted to generate the amplicons of claim 5(d) and claim 5(e) respectively, and then identifying the microbe as *G. lamblia* in the case where both amplicon(s) are detected.

17. A method for discriminating between the assemblage A and assemblage B genotypes of *G. lamblia*, comprising the step of subjecting DNA extracted therefrom to first and second polymerase chain reactions using (1) the primer and probes of SEQ ID NO.s 13, 14 and 15, and (2) the primer and probe sets of SEQ ID NO.s 19, 20 and 21, and then identifying the genotype as assemblage A in the case where the primer and probe set (1) produces a detectable amplicon.

18. A method according to claim 1, wherein the extracted DNA is treated, prior to amplification, with at least one agent to reduce inhibitors of a polymerase chain reaction.

19. The method according to claim 18, wherein the agent includes a binding agent selected from an ion chelator and a protein scavenger.

20. A method according to claim 1, adapted for detection of DNA extracted only from viable cells.